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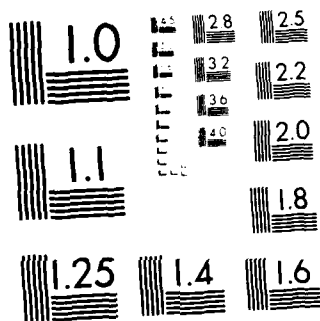
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ABSTRACT

With respect to plague potential, Vandenberg Air Force Base is considered to be one of six high risk military installations in the Western U.S. A survey of "free-living" fleas in ground squirrel burrows on Vandenberg Air Force Base was conducted during the period 1977-1980 as part of a cooperative U.S. Air Force-California plague surveillance program. Diamanus montanus was the numerically dominant flea throughout the study period. Dominant populations of Hoplopyllus anomalus were not observed during the summer months as is recorded for other areas of the state. It is hypothesized that the cool, moist coastal summer climate may be a major ecological factor which depresses the Hoplopyllus population. The fact that Diamanus sp. predominates throughout the year is noteworthy as it is the most viable vector for plague transmission among the ground squirrel populations and to humans interfacing with the ground squirrel habitat. Laboratory cultures of pooled fleas for Yersinia pestis were always negative, however, serological surveys of wild mammals from the base have generated twelve positive plague titers since the program's inception in 1977. Major die-offs of ground squirrels have not occurred on the base within the recent past. Proposed future studies will add raw data from years 1981 and 1982 to the existing data-base and correlate environmental variables (weather, geo-magnetic activity) to temporal and spatial densities of ground squirrels and associated fleas.

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Plague Epidemiological Surveillance, Vandenberg Air Force Base, California:

Part I. Descriptive Report of the Surveillance Program with Special
Emphasis on Extracorporeal Fleas from Ground Squirrel Burrows

by

Larry D. Brown, B.S., D.V.M., Major
Air Force Institute of Technology
United States Air Force

Submitted in Partial Fulfillment
of the Requirements for the Degree of
MASTER OF PREVENTIVE VETERINARY MEDICINE
(MPVM)

Department of Epidemiology and Preventive Medicine

School of Veterinary Medicine
UNIVERSITY OF CALIFORNIA, DAVIS
California 95616 U.S.A.

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Reviewed and Approved by

M.M.J. Lavoipierre
Professor of Parasitology

M. Pappaioanou
Assistant Professor of Epidemiology
and Statistics

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DEDICATION

I wish to dedicate this paper to my wife Linda and sons Rick and Tony for their love, understanding and assistance during the past year.

ACKNOWLEDGEMENTS

- I wish to thank: 1) The U.S. Air Force for sponsoring my one year educational tour at UC Davis.
- 2) All the Air Force veterinarians and technicians who collected field data on ground squirrel and flea populations on Vandenberg Air Force Base, California during the years 1977-1982. A special thanks is owed Major (Dr.) Steve Grube and Senior Airman Murray who gave considerable time and assistance during my trip to Vandenberg Air Force Base and to Major (Dr.) Rick Somers and SMSgt Washington for initiating collection of field data.
- 3) Dr. Bernard L. Nelson, Senior Vector Biologist and Staff, State California Department of Health (VBCB), Berkeley for providing: inspiring lectures on plague in the MPVM program, the assembled raw data forms, much of the original guidance for this paper and flea identification services at the State Laboratory for specimens collected by Vandenberg AFB medical technicians.
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- 6) Dr. Walter E. Howard of the UCD Division of Wildlife and Fisheries Biology and Dr. Jameson of the Zoology Department for their assistance and expertise on ground squirrels and fleas.

INTRODUCTION

Vandenberg AFB (VAFB) is a large U.S. Air Force (USAF) installation located on the pacific coastline in Santa Barbara County, California. It is currently the training center for USAF ballistic missile crews and a launch point for space satellites. Vandenberg is slated for an increase in mission responsibilities during the eighties as it will become the launch and recovery point for the U.S. Space Shuttle Program. The Beechey or California Ground Squirrel, Spermophilus beecheyi (Richardson) is abundant in many areas of the installation. It is estimated that 787,000 ground squirrels inhabit the installation (Table 1). Ground squirrels have been involved in documented epizootics of sylvatic plague in California for many years (1). Vandenberg AFB, and the Santa Barbara County area, are considered by state public health authorities to be one of several ecological foci of plague in the state (2). As far as can be ascertained from records, the last case of human plague in Santa Barbara County occurred in 1928(2,3). Major die-offs of ground squirrels have not occurred on the base within the recent past. Plague control, including education and rodent control, has been practiced on the installation for many years (3).

In 1976 the Deputy for Environmental Affairs of Department of Defense, in cooperation with the USDHEW/PHS Centers for Disease Control (CDC), state and local authorities, established formal plague surveillance programs on thirty-two Department of Defense installations in the Western United States (west of 100th meridian). These programs were implemented in Utah, Colorado, Oregon, Wyoming, Arizona, New Mexico, Nevada and California because of the increasing number of civilian cases of human

plague reported from the Center for Disease Control, a plague fatality that occurred at Kirtland AFB, NM in 1976 and the increasing widespread evidence in and die-offs of wild rodent (sylvatic plague) vectors in western states (5,6). Arizona and California now rank second and third, respectively, to New Mexico in annual numbers of human plague cases and sylvatic epizootics (7). Although the organism (Yersinia pestis) responsible for plague has been well studied, much of the ecology of sylvatic plague cycles, the study of flea population dynamics, the identification of definitive reservoir rodent species and geographical foci and development of exact epidemiological and mathematical risk assessment models are still in early stages of development. Whilst many animal and human diseases readily follow host-agent-environment predictive models, the ecology of plague as a disease contains yet another determinant, an arthropod vector, which complicates attempts at modeling this disease. It is, nonetheless, a well accepted premise that flea biology and population dynamics play an important role in the vectoring of plague and in the epizootics seen in indicator or natural sentinel species (8,9,10).

Stark (11) and Pollitzer (12) reported Diamanus montanus as one of a list of sixty-one fleas which may be important in the epidemiology of plague in the United States (Table 2).

The California Ground Squirrel and the flea Diamanus montanus were first implicated in the epidemiology of sylvatic plague in 1908 (13, 14, 15). The ground squirrel then achieved notoriety as one of three important primary reservoirs of plague in the western United States (16). It is now generally accepted that smaller rodents, such as the Microtus and Peromyscus, act as the primary reservoir of plague in California and

that the intermittent epizootics of the disease in the "natural sentinel" ground squirrel are originated by transfer of infected fleas from the relatively resistant reservoir hosts to the ground squirrel (9,13,17, 18,19, 20).

In 1914 Bacot and Martin conducted classical experiments on the mechanism of the transmission of plague by fleas. Xenopsylla cheopis and Nosopsyllus fasciatus were the two flea species observed. In the U.S. Eskey (1938), Eskey and Haas (1940), Douglas and Wheeler (1943), Burroughs (1947), and Holdenried (1952) have experimentally studied flea species other than X. cheopis and N. fasciatus and showed that many other flea species are efficient plague vectors (21). In 1944 Diamanus montanus was found to be a more efficient vector of plague than Xenopsylla cheopis (22). In a 1943 epizootic of plague among ground squirrels in Kern County, CA, Hoplopsyllus anomalous was demonstrated to be naturally infected but not an efficient vector of plague in the laboratory (13, 22, 23).

More recently studies by Holdenried (1952) has shown Diamanus to be a very potent vector of plague (24). This is explained by the Diamanus species accelerated rate for proventricular "blocking" when Yersinia organisms multiply within its alimentary canal. Blockage causes the flea to become hungry and dehydrated, and both its mobility and frequency of feeding attempts increase (25).

It is noteworthy that the ability of fleas to become blocked with Y. pestis (26) and to transmit the agent (26, 27, 28) declines as environmental temperatures exceed 27.5°C (29).

The World Health Organization (WHO) reports four major types of flea population indices (30): 1) the total flea index (average number of fleas

of all species per host); 2) the specific flea index (average number of fleas of each species per host); 3) the burrow or nest flea index (average number of free-living fleas of each species per burrow or nest) and 4) the house flea index (average number of Pulex irritans, Synosternus pallidus, or other species of flea per house).

The plague literature lacks recent published reports on California Ground Squirrel burrow flea indices; most reports pertain to on-host (total or specific flea index) measurements.

The surveillance data collection on which the present study is based was initiated by USAF Hospital Vandenberg in May 1977 and continued to the present time.

The objectives of this study are 1) to describe ecological setting and weather conditions of Vandenberg Air Force Base; and 2) to provide information on ground squirrel burrow flea (free living or extracorporeal) density measurements and seasonal population patterns at a coastline location in California.

ECOLOGICAL DESCRIPTION OF STUDY AREA

An excellent description of the local ecology and weather for the Vandenberg area was provided by Pinkovsky in 1980 (31). It is quoted here verbatim because the present author feels that this description cannot be improved upon. The accompanying maps do not belong to the original text by Pinkovsky but have been added by the present author.

"Vandenberg AFB is a Strategic Air Command installation located in Santa Barbara County (Figure 1*) on the south central California coast approximately 150 miles northwest of Los Angeles. The city of Santa Maria lies just to the northeast of the base, and the town of Lompoc is adjacent on the southeast. The base is the third largest USAF installation in the United States encompassing 98,400 acres along 35 miles of Pacific coastline (Figure 2**). The base is headquarters for the First Strategic Aerospace Division and the Space and Missile Test Organization and serves as the launching center for test firing of operational land-based intercontinental ballistic missiles and polar orbiting space satellites. During the decade of the 1980s, Vandenberg AFB will be the focal point for the Space Shuttle and MX Missile testing programs. The elevation of the base varies from sea level to 2,135 feet at Tranquillon Peak in south Vandenberg. The main cantonment area in north Vandenberg encompasses about 6,000 acres of low-lying plateau at 400 feet elevation. Drainage in north Vandenberg is through the San Antonio

*Figure 1 Source: Vandenberg AFB with modification by L. Brown.

**Figure 2 Source: Vandenberg AFB with modification by L. Brown.

Creek watershed; vegetation in this area of the base includes riparian vegetation, scrub oak woodlands with introduced Eucalyptus and Bishop pine, and annual grasslands. South Vandenberg, below the Santa Ynez River, consists of steep canyons and ridges with most vegetation in the natural state. Chaparral, grasslands, and Bishop pine and tanoak forests predominate. Ground squirrels, gophers, field mice, California mule deer, feral pigs, bobcats, predatory birds, and waterfowl are just a few of the extensive wildlife species found on the installation. Grazing and agricultural outgrants affect 37,500 acres: 21,000 acres are used by the Federal Correctional Institution to graze cattle and raise crops and 16,500 acres are outleased to private cattle ranchers. Six endangered plant species and four endangered animals--the California Least Tern, California Brown Pelican, the Peregrine Falcon, and the unarmed, three-spine Stickleback--occur on or range over the base. The base supports over 28,000 people including active duty military, dependents, civilians, contractor personnel, and retirees. Recreational areas on Vandenberg AFB include family campgrounds, beaches, picnic areas, a swimming pool, playgrounds, ball fields, saddle club, tennis courts, 18 hole golf course, and jogging trails. The base has an active scouting program, each summer hosts ROTC summer encampments, and is frequently the site of PRIME BEEF* exercises.

Annual rainfall averages 13 inches, 95% of which occurs during November through April. During the dry summer, dense morning and evening fog is a common phenomenon experienced on an average of 15 days each month. Due to the Pacific Ocean's moderating influence, temperatures

*PRIME BEEF: A name given to military exercises carried out by USAF Civil Engineer Squadrons.

are fairly constant year round. Ten months out of the year the average maximum temperature is 60-65°F, and the average low temperature is in the 40-50°F range. September and October are the warmest months with an average high temperature of 69°F."

MATERIALS AND METHODS

Data Collection

The Vandenberg AFB Veterinary Services, in close cooperation with the California Department of Health, Vector Biology Section, implemented an intensive sylvatic plague host and vector surveillance program in 1977. This program collected five types of data: mammal serology, ground squirrel counts, burrow counts with measure of activity, flea counts, and flea cultures. Raw data was stored at the Vector Biology Division of the California State Department of Health.

Serology

Vandenberg AFB Veterinary Services, with the assistance of state employees, performed serologic epidemiology studies for plague in carnivores and rodents during 1979-1981. Sera from coyotes (Canis latrans), dusty-footed rats (Neotoma fuscipes), deer mice (Peromyscus maniculatus), California pocket mice (Perognathus californicus), badgers (Taxidea taxus), bobcats (Lynx rufus), meadow mice (Microtus californicus) and California ground squirrels (Spermophilus beecheyi) were collected.

Ground Squirrel Counts

Since May 1977, VAFB veterinary staff made monthly or bi-weekly field surveys on populations of Spermophilus beecheyi at specified plots of standard size (50 X 100 yards) to determine changes in population size and distribution, to detect die-offs and to count burrows and collect fleas. Stationary survey sites (SSS) or flea collection plots included beach area or sand dunes (3 plots: sites S-7, S-8, S-9; see Figure 2), cantonment* area (12 plots: sites S-2, S-2A, S-2B, S-3, S-4, S-4A, S-4B,

*Cantonment: 6,000 acre area where base housing and base industrial complex is located. Human activity and density is greatest here. Cantonment is represented by Figure 3. Source: Vandenberg AFB with modification by L. Brown.

S-5, S-6, S-10, S-11, S-12; see Figure 3) and golf course area (1 plot: site S-1; see Figure 2). Additionally, five ground squirrel road count survey (RCS) areas, four woodrat den survey (WRS) areas and five ground squirrel visual site survey (VSS) areas were also visited during portions of the 1977-1980 period (see Figure 2 and 3). Data collection for the latter three types (RCS, WRS, VSS) of surveys was limited to mammals (i.e. flea specimens were not collected).

Burrow Counts

At the sixteen stationary sites, an observational estimation of burrow prevalence and activity (active or abandoned) was determined by veterinary medical technicians following a standard operating procedure (32).

Flea Counts and Cultures

Samples of fleas were collected from active ground squirrel burrows at each of the sixteen sites or plots by "flagging" (insertion of ether soaked 1 ft. sq. flannel cloth into burrow) fifteen burrows to a standard depth of one foot. Fleas were counted, identified and sexed at the CA State Department of Health Laboratory, and fleas from each site were pooled and cultured for the presence of Yersinia pestis at the USPHS Plague Lab in Ft. Collins, Colorado. Teneral (young) fleas were never submitted in pools. Raw data was placed on original field survey forms (Exhibit 1) at VAFB and VBCB, Berkeley for the period 1977-1980. The method by which the 15 burrows were randomly selected for flagging was not reported.

Data Collation

A total of 967 field survey forms (Exhibit 1) containing up to a maximum of thirty variables each was coded by the author at UC Davis. An

example coding sheet is provided as Exhibit 2. The coded data was entered, chronologically as collected, into the campus Burroughs 7800 computer by the UC Davis Computer Department Key punch Services personnel. Computer files were verified prior to analysis. The data base was first computer sorted chronologically by survey site code. Two hundred ninety-eight surveys not containing flea data (area codes RCS, WRS, VSS) were removed to other files. Data for the stationary site (SSS) areas was adjusted to remove surveys: 1) where fleas were not collected because of windy conditions or manpower shortage; 2) where fleas collected were lost in mail or were in deteriorated condition precluding identification on arrival at laboratory; 3) where concurrent rodent control measures were in effect at time of survey. Four hundred ninety-two surveys of data remained out of the original 669 for descriptive and statistical analysis.

Methods of Analysis

Minitab (33) and BMDP (34) statistical packages were used to analyze the data on the B7800. BMDP7D was run to obtain basic descriptive statistics on flea counts for the sixteen areas surveyed.

RESULTS

The results of this study is divided into three sections: Survey Descriptive Data, Mammal Serology, and Flea Descriptive Data.

Survey Descriptive Data

During the period 1977-1980, 967 total field surveys for surveillance of potential plague hosts (ground squirrels and woodrats) and vectors (fleas) were carried out on the confines of Vandenberg AFB, California (Table 3). Sixty-nine percent (69%) of these surveys included collection of flea data. Remaining surveys included 263 ground squirrel road counts, 27 woodrat den inspections, 5 visual site surveys and 3 surveys noted as "others". Surveys were accomplished by eleven different Air Force veterinary medical technicians during the period 1977-1980 (Exhibit 3). The number of surveys conducted by each inspector ranged from a low of 7 to a high of 245 surveys. It is noteworthy that four inspectors conducted 742 or 76.73 percent of all surveys (Table 4). One hundred twenty-four surveys were conducted by one inspector while the remainder were conducted by a team of two inspectors.

The time-of-day distribution for the 967 surveys is expressed in military hours in Table 5. Five hundred ninety-nine or 61% of 967 surveys were conducted between 0800 and 1200 hours. Twenty surveys did not report time-of-day. The remainder of the surveys were accomplished during the afternoon (1200-1600) hours.

During the 967 surveys, 7,937 ground squirrels were counted, an average of 8.21 per survey (Table 6). Burrows counted amounted to 28,645 or an average of 29.62 per survey. The majority or 19,986 (69.77%) of

these burrows were active and 8,659 (30.23%) were inactive. A total of 10,035 active burrows were flagged (swabbed) for fleas. Total fleas collected amounted to 6,172 over the four years or an average of 9.23 fleas per survey.

Mammal Serology

One hundred sixteen rodent and forty-nine carnivore sera samples were analyzed by the USPHS Plague Laboratory, Ft. Collins, Colorado during the period 1979-1980. To date twelve positive indirect passive hemagglutination (IPHA, IHA or IDA) titers for Yersinia pestis have been recorded for wild mammalian species on VAFB (Table 7,8,9).

Flea Descriptive Data

Flea data was not collected consistently on all sixteen stationary site survey (SSS) areas for all of the four years of this study. During 1977 seven areas were under survey. Five new areas were added in 1978 and four new areas were added in 1979 to give a total of sixteen study areas. Two areas were dropped in 1980. Within each year data was not always collected for each month of the year (Table 10).

Numerous* flea pools were analyzed by the USPHS Plague Laboratory, Fort Collins for presence of Yersinia pestis. To date all cultures have been negative.

Nine genera of fleas were identified from the pool of 6,172 fleas collected during the four years, 1977-1980 (Table 11). Diamanus (DM) species was the numerically dominant flea in the study representing an average of 93.68% of the total fleas collected, and ranged from a low of

*Between 1977-1980 one hundred seventy-five flea pools containing 1920 total fleas were cultured. All were negative.

59.78% in Area 3 to a high of 99% in Area 14. Hoplopsyllus (HA) species represented 4.19% of the total fleas collected and ranged from a low of 0.00% in Area 16 to a high of 35.86% in Area 13 (Table 12). Surveys during years 1979 and 1980 produced greater numbers of Diamanus fleas and 1979 was the high year for Hoplopsyllus collections (Table 11).

Years 1977 and 1979 actually had the largest average number of fleas per survey (Table 13). Areas four and eight had the largest apparent mean number of fleas collected per survey as well as the largest variance (Table 14). Variance (STD. DEV.²) or dispersion within groups or study areas was quite large (Table 14). Statistical significance of the difference between groups will be determined during future studies.

Echidnophaga sp. were identified only in year 1977 and 9 of 12 found came from one site (Area 1 or S1, Table 11). The remaining five genera (Pulex, Monopsyllus, Malaraeus, Opisodasys, Atyphyloceras) of fleas identified are considered casual species or incidental to this study as they do not have host preference for the California ground squirrel, but rather meadow, deer or field mice and the wood rat. The presence of these flea species in ground squirrel burrows is a normal, not infrequent, finding confirming the cohabitation of burrows and study plots by other types of rodents (13,25).

The sex ratios (male:female) for the three major genera of ground squirrel fleas collected is reported in Table 15. During the four year period, the female Diamanus slightly outnumbered the male, the male Hoplopsyllus outnumbered the female and the Echidnophaga species were found in equal numbers of both sexes. Sex ratios (M:F) were 1:1.099, 1.14:1 and 1:1 respectively with an overall ratio of 1:1.088. Sex ratios by year were not calculated, however, the raw data for these calculations is available in our databank.

DISCUSSION

Comparative studies of seasonal patterns for populations of Diamanus montanus, the ground squirrel or "winter flea" and Hoplopsyllus anomalus, the "summer flea" on the California Ground Squirrel have been reported for various inland locations in California (13,25,35,36,37,38,39). A search of the literature revealed one prior study of seasonal patterns for a coastal location in Orange County, Ca (39). The normal flea population density seasonal pattern for valley, mountainous area and one coastal location in California appears to be cyclic in nature with a short-term winter-summer inversion of the number of Diamanus and Hoplopsyllus fleas on ground squirrels and free-living in burrows. Diamanus predominates during the winter or cool moist period (mean temperature below 75°F) and Hoplopsyllus predominates during the summer or dry season (mean temperature above 75°F) (37,39; see Table 16). Lack of moisture is the limiting factor restricting D. montanus breeding in summer. H. anomalus fleas are reproductively inhibited in summer and winter by rainfall in an amount to moisten the soil to 1 meter in depth (40).

The most important finding of this study was that the typical seasonal flea population pattern for California was not evident at Vandenberg AFB. The winter-summer inversion was not observed and Diamanus montanus was the predominate flea throughout the study period (1977-1980, Table 17). Contrary to the typical California pattern, Hoplopsyllus anomalus was not numerically dominant at any time during the year including the summer periods when this species normally predominates in other areas of the state (Table 17). These preliminary findings suggest that the coastal climatic conditions on VAFB may be an important

determinant of the flea populations located there. This 'Vandenberg pattern' was predicted by Dr. B.C. Nelson, State of California Department of Health Vector Biologist, prior to this study (i.e. the temporal density of Diamanus and Hoplopsyllus flea populations on VAFB would differ when compared to known regions of the state because of the coastal climatic conditions).

Weather on VAFB may also be important with regards to vector potential and vector capacity of fleas infected with Y. pestis. Data published by Kartman and Prince (1956), Pollitzer (1954) and Kartman (1969) confirm that there is an optimum temperature for which a flea may retain its infection, for an extrinsic incubation period, for formation and proper location of a Y. pestis mass or plug, for flea longevity, for success of the proventricular blocking mechanism and finally, for a flea's vector capacity. This optimum temperature is 23.5°C (74.3°F) for X. cheopis (26). In general, the higher the temperature the less likelihood that the fleas would reach the infective state (26). Cavanaugh and Williams reported in 1980 that a flea's ability to transmit the agent Y. pestis declines as environmental temperatures exceed 27.5°C (81.5°F) (29). Optimum temperatures for Diamanus montanus have not been reported; however, let us suppose that it would not differ greatly from that of X. cheopis. The VAFB average maximum temperature is 60-65°F for ten months of the year. The average low temperature is in the 40-50°F range. September and October are the warmest months with an average high temperature of 69°F. These temperatures are conducive to supporting an epizootic on VAFB at most anytime of the year during which the disease might be introduced. The winter

estivation period of the California ground squirrel on VAFB would be another factor which would reduce the likelihood of an epizootic during winter months (36). Additional environmental and ecological factors are undoubtedly involved as well (41).

Numerically the two most important fleas collected on VAFB were Diamanus montanus and Hoplopsyllus anomalus. Additionally Echidnophaga sp and six casual species of fleas were recovered from burrows (Table 11). Of the casual species of fleas collected from VAFB ground squirrel burrows, Malaraeus telchinum, Atyphloceras multidentatus and Monopsyllus wagneri have been implicated in the epizootiology of sylvatic plague in the Western U.S. (17,20,42). The importance of these fleas in vectoring plague on Vandenberg AFB must be slight because of the small numbers in the ecosystem. Echidnophaga gallinacea, Pulex simulans and Opisodasys (keeni) nesiotus are less well known for vectoring of plague.

Only twelve E. gallinacea specimens were collected from burrows on VAFB during the four years of this study (Table 11). In one study E. gallinacea was reported as the numerically dominant flea in southern California during the months of June through October (38,39). Studies conducted in San Bernardino County, CA, during 1952 and 1953 have indicated that the index of E. gallinacea is adversely affected by cool weather associated with fall or early winter rains (19,20). Because of this "sticktight" fleas feeding habits (25) and the extremely small population on VAFB it is unlikely to be a significant vector of plague on VAFB.

Malaraeus telchinum, a poor vector of plague in the laboratory, appears to be instrumental in maintaining enzootic plague among California meadow mice (43).

Opisodasys (keeni) nesiotus and Catallagia wymani, two North American wild rodent fleas found on the widely distributed deer mouse, Peromyscus maniculatus, are virtually restricted to foggy coastal areas (43).

It is now suspected that fleas of the genus Catallagia sp. are important in vectoring of plague from asymptomatic resistant latent carrier rodent populations to populations of the highly susceptible ground squirrel in California (45). Catallagia sp. were absent from the pool of fleas collected on VAFB during the period 1977-1980. This key vector absence may partially explain why the disease has not apparently spilled over from reservoir rodent populations into ground squirrel populations. Plague epizootics have not occurred amongst the ground squirrel population on Vandenberg AFB within the past 10 years (4,45).

Based on the above findings the following human health risk assessment is offered. Although definitive risk assessment formulas are unavailable, it appears that Vandenberg AFB fulfills many of the requirements for a high risk installation. There are large numbers of ground squirrels and potent vectors (Diamanus sp.) within the 6,000 acre cantonment area where human activity is greatest (Table 1,6,11). Latent plague (Y. pestis) is present in wild reservoir rodent species and sentinel coyote species on the installation (Table 7). The laboratory method used for the serology studies (i.e. IPHA) is the most accurate of the four major serological tests available today (Table 18) (44). The cool, moist weather along the coast favors a potent flea vector, Diamanus sp. (37,39,40). The weather may also encourage "blocking" which stimulates a flea's urge to move and feed and increases the potential for transmission among fleas which feed on infected rodents (29).

From the opposite perspective, one could state that despite the presence of these risk factors, human plague has not been reported on the installation. Also, major dieoffs of the ground squirrel have not occurred recently, and education programs are intensive and effective. As well the Veterinary Inspectors* keep a close watch over the sentinel ground squirrel population, pest control personnel are prepared to implement emergency ground squirrel and flea control measures and medical officers normally have a high "index-of-suspicion" for plague. In combination with the above factors present day nutrition, diagnostic tools, antibiotics and surveillance reduces the likelihood that any human outbreak (one or more cases) would gain a major foothold. The appearance of even one publicized human case or death on VAFB may have great psychological impact for a short period because of the effect plague has had on human history.

Future data collection requires some improvements. It was apparent that flea data was not collected consistently on all sixteen stationary survey areas during the four years of this study (Table 10). This finding could compound planned future efforts at time-series analysis of this database. This may be overcome at Vandenberg by placing greater emphasis on consistently surveying each area on a monthly basis. Areas should be carefully chosen before study start-up begins and efforts made to keep these areas in the surveillance program. Manpower constraints may require that fewer areas be selected for the study; however, once an area is chosen it should remain in the study for at least three years and longer if feasible. One recent University of California time-series

* 1981 DOD Plan for Reorganization of USAF Veterinary Services changed the name of the service and the technicians to Environmental Health Services and Environmental Health Technician respectively.

analysis study of Microtine rodent populations analysed a database containing 19 years of data (46). In view of these minor criticisms, the author feels compelled to note that the USAF Hospital Vandenberg Environmental Health Services, with the cooperation of the Vector Biology Section of the California State Department of Health and the USAFSAM Epidemiology Division has established one of the finest plague surveillance and data collection systems in the DOD and the U.S. Kirtland AFB, NM, Fort Ord Complex, CA and various states and universities also have strong ongoing plague surveillance programs.

Surveys during years 1979 and 1980 produced greater numbers of Diamanus fleas and 1979 was the high year for Hoplopsyllus collections (Table 11). This does not mean; however, that the burrow densities were greatest during those times as greater numbers of surveys were accomplished during 1979 and 1980 (Table 3). Years 1977 and 1979 actually had the largest flea population densities (Table 13).

Time-of-day of data collection is an important consideration as fleas have daily activity periods (25) and these periods could present greater numbers of fleas at the burrow openings during certain times of each day. Time-of-day of technician surveys could affect the densities measured especially if one technician assigned duty for one month likes to survey early AM hours and the technician for the following month prefers to survey during afternoon hours. Sixty-one percent of all surveys were conducted between 0800 and 1200 hours. This may slant the database towards the AM period and cause excessive or subnormal flea counts to be obtained depending upon whether ground squirrel fleas

prefer to frequent the mouth of the burrow during the morning or afternoon. This is unknown at this time and is probably related to host activity as well. Daily flea activity periods could significantly affect the analysis of this database and cause false conclusions to be drawn. Future analysis of this data will include description of daily flea activity.

A major question was raised early during the conduct of this study on the correlation between on-host and extracorporeal or "free-living" counts of fleas. Review of the literature provided one reference to this question. Flea density and species composition at the mouths of burrows reflects with a high degree of accuracy, but on a smaller magnitude, the population density and species composition on the host (37).

The number of surveys for the 16 base areas studied ranged from 6-58 surveys (Table 3). The average number of surveys was 30.75. The 1977-1980 database is sufficiently large to enable biologically meaningful conclusions to be drawn. Expanding the database to include 1981 and 1982 years should improve on the statistical validity of any conclusions drawn from future studies.

Surveys where concurrent rodent control measures were in-effect at time of survey were removed from the original database prior to analysis (compare Tables 12 and 14). This amounted to removal of 78 surveys. This was determined necessary as ground squirrel control measures may cause high numbers of "orphan fleas" to be present at the burrow opening awaiting a new host. Baseline state-of-nature determinations are next to impossible when human activity affects the ecosystem under study. For this reason ecological studies of this type are best

accomplished on areas exempt from any type of human generated factors or as the second best option areas under standardized management practices. Vegetation mowing practices, ROTC training on fields next to study plots, predation, disease, human activity, etc. are other unavoidable environmental factors which may affect the counts of ground squirrels or fleas. Inspectors should continue to document any of these observations while conducting field surveys.

It is recommended that future analysis of the Vandenberg database include Analysis of Variance (ANOVA) to compare study areas or to identify areas of greatest flea or ground squirrel density, Time Series Analysis to determine seasonal variation, long-term secular trend, or cyclical fluctuations, and Isodemic Mapping to determine spatial densities of fleas and ground squirrels.

It is the authors personal opinion that continued strong surveillance is warranted and building upon baseline data should be encouraged. This data can be useful in fighting epizootics, in planning control measures, for health education programs and for preparation of environmental impact statements. Surveillance is the key to early detection of any animal or human epidemic and it enables health officials to nip episodes in the bud before they become epidemics. Suggested goals are provided as a focus for this program (Table 19).

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TABLE 1

Estimation of Ground Squirrel Numbers on Vandenberg Air Force Base, CA

- I. Squirrel Density, 1977-1980: Average of 8 squirrels observed at any given time or season of year on survey areas (50 x 100 yds).
- II. Size Survey Plot: Acre = 43,560 sq. feet; 208.71 ft sq = 1 acre; 50 x 100 yd area = approx. 1 acre.
- III. Total Base Acreage: 98,400.
- IV. Total Squirrel Numbers on Installation: $98,400 \times 8 \approx 787,000^*$ if distributed evenly over installation as on 16 survey areas.

*This figure could be reasonably reduced by 2% to account for lawns, golf course, fixed facilities, pavements and the runway. Ground squirrels would not be expected to inhabit these grassy areas or paved or concrete surfaced acreage; however, one colony at the Minuteman Beach Site (Site S-8, Figure 2) has undermined the roadway. As a vehicle approaches, the squirrels run to the middle of the road and disappear into a 1½ ft diameter burrow opening into the center of the blacktop roadway. The sandy soil beneath the roadway has apparently encouraged this unusual location for a burrow system.

TABLE 2

A List of Fleas in the United States Which May be Important in the Epidemiology of Plague

Modified and rearranged in alphabetical order after Pollitzer, 1960, Bull. WHO, 23: 397-400 and Stark, 1958, USDHEW, PHS, CDC: 2-5.

N - <u>Anomiopsyllus species</u>	TEN - <u>Orchopeas sexdentatus</u>
N - <u>Anomiopsyllus hiemalis</u>	T - <u>Orchopeas sexdentatus sexdentatus</u>
EN - <u>Anomiopsyllus nudatus</u>	EN - <u>Oropsylla idahoensis</u>
TEN - <u>Atyphloceras multidentatus</u>	T - <u>Oropsylla rupestris</u>
N - <u>Atyphloceras species</u>	N - <u>Peromyscopsylla hesperomys adelpha</u>
ENP - <u>Catallagia decipiens</u>	N - <u>Pleochaetis sibynus</u>
E - <u>Catallagia wymani</u>	T - <u>Polygenis gwyni</u>
T - <u>Ctenocephalides canis</u>	TEN - <u>Pulex irritans</u>
TEN - <u>Ctenocephalides felis</u>	N - <u>Stenistomera alpina</u>
TEN - <u>Diamanus montanus</u>	N - <u>Stenistomera (Miochaeta) macrodactyla</u>
TEN - <u>Echidnophaga gallinacea</u>	T - <u>Thrassis acamantis howelli</u>
N - <u>Epitedia stanfordi</u>	T - <u>Thrassis acamantis utahensis</u>
TEN - <u>Epitedia testor</u>	TEN - <u>Thrassis arizonensis littoris</u>
TEN - <u>Epitedia wenmanni</u>	TN - <u>Thrassis bacchi gladiolis</u>
N - <u>Foxella ignota</u>	NPT - <u>Thrassis bacchi johnsoni</u>
EN - <u>Hoplopsyllus glacialis</u>	N - <u>Thrassis fatus</u>
	TEN - <u>Thrassis francisi</u>
TEN - <u>Hoplopsyllus anomalus</u>	ET - <u>Thrassis pandorae</u>
TN - <u>Hystrichopsylla dipptei</u>	EN - <u>Thrassis petiolatus</u>
	TEN - <u>Thrassis stanfordi</u>
TEN - <u>Hystrichopsylla linsdalei</u>	TEN - <u>Xenopsylla cheopis</u>
E - <u>Leptopsylla segnis</u>	
TEN - <u>Malaraeus telchinum</u>	
TEN - <u>Megabothris abantis</u>	
NP - <u>Megabothris clantoni</u>	
N - <u>Megabothris clantoni</u>	
E - <u>Megarthroglossus divisus</u>	
NP - <u>Meringis shannoni</u>	
E - <u>Monopsyllus ciliatus</u>	
TEN - <u>Monopsyllus eumolpi</u>	
TEN - <u>Monopsyllus exilis</u>	
TENP - <u>Monopsyllus wagneri</u>	
EN - <u>Neopsylla inopina</u>	
TEN - <u>Nosopsyllus fasciatus</u>	
T - <u>Opisocrostis bruneri</u>	
TEN - <u>Opisocrostis hirsutus</u>	
TEN - <u>Opisocrostis labis</u>	
N - <u>Opisocrostis t. cynomuris</u>	
TEN - <u>Opisocrostis t. tuberculatus</u>	
TN - <u>Opisodasys keeni nesiotus</u>	
N - <u>Orchopeas leucopus</u>	
N - <u>Orchopeas neotomae</u>	

T - Known to transmit in laboratory.

E - Experimentally infected in laboratory.

N - Found naturally infected.

NP - Naturally infected in a pooled inoculation with other fleas.

TABLE 3

VAFB Plague Surveillance Surveys, CY 1977-1980

Surveys by Year and by Survey Type

Type Survey	Numbers Conducted by Period				
	1977	1978	1979	1980	1977-1980 Overall
All Types	124	185	244	414	967
Stationary Sites (SSS)	67	108	160	334	669
Road Counts (RCS)	27	48	73	115	263
Woodrat Den Inspection (WRS)	27	0	0	0	27
Visual Site (VSS)	0	0	0	5	5
Others*	0	0	3	0	3

*Areas designated by codes: J2A(1), M3LK(1), SLC3(1)

TABLE 4
Number Surveys by Inspector**Code

967 Surveys, 1977-80

<u>Code</u>	<u>Number</u>
1	155
2	177
3	245
4	37
5	33
6	49
7	13
8	12
9	65
10	165
11	7
Inspector Unknown *	9
ALL	967

*Inspector names were not reported on 9 surveys.

**One hundred twenty-four surveys were conducted by one technician.
Remaining surveys were conducted by a team of two technicians.

TABLE 5

Time of Survey Distribution

967 Surveys, 1977-80

	Middle of Interval	Number of Observations	
	0.	20	** (1)
	200.	0	
	400.	0	
	600.	0	
Time of Day	800.	240	*****
(Military Hours)	1000.	359	*****
	1200.	119	*****
	1400.	193	*****
	1600.	36	****
	1800.	0	
	2000.	0	
	2200.	0	
	2400.	0	

(1) Surveys where time was not reported were counted as zeros.

TABLE 6

California Ground Squirrel, Flea and Burrow Data Collected During 967
Field Surveys, Vandenberg AFB, CA, 1977-1980

Category	Total Number (% of total)	Average/ Survey
Ground Squirrels Counted	7,939	8.21
Burrows Counted	28,645	29.62
Active Burrows	19,986 (69.77%)	20.67
Inactive Burrows	8,659 (30.23%)	8.95
Burrow Surveys for Fleas	669 (69.18%)	N/A
Burrows "Flagged"	10,035	15
"Free-Living" Fleas Collected, All Species	6,172	9.23

TABLE 7
 Plague Surveillance: Positive Serological Test Data
 Vandenberg AFB, CA, 1979-1982

Positive Serologies	Approximate Location	Date	Titer
1. <u>Canis latrans</u> (coyote)	Destroyer Point	26 Jul 79	1:256
2. <u>Canis latrans</u> (coyote)	Destroyer Point	11 Oct 79	1:64
3. <u>Canis latrans</u> (coyote)	2 mi W of Casmalia	20 Oct 79	1:128
4. <u>Canis latrans</u> (coyote)	Spring Canyon	24 Jan 80	1:2048
5. <u>Microtus californicus</u> (meadow mouse)	Spring Canyon	5 Jun 80	1:128
6. <u>Peromyscus maniculatus</u> (deer mouse)	Spring Canyon	5 Jun 80	1:256
7. <u>Neotoma fuscipes</u> (dusty-footed woodrat)	Picnic Grounds	Nov 80	1:1024
8. <u>Neotoma fuscipes</u> (dusty-footed woodrat)	Picnic Grounds	Nov 80	1:128
9. <u>Canis latrans</u> (coyote)	1 mi W of Penitentiary	Feb 81	1:256
10. <u>Peromyscus maniculatus</u> (deer mouse)	Spring Canyon	28 Jul 81	1:256
11. <u>Canis latrans</u> (coyote)	3 mi SW of Orcutt	2 Feb 82	1:256
12. <u>Canis latrans</u> (coyote)	4 mi SW of Orcutt	19 Apr 82	1:256

TABLE 8
Wild Rodent Serological Surveys for Plague
Vandenberg AFB, California, 1979-82

	1979	1980	1981	1982	Total
Total Rodents Surveyed	0	66	50	0	116
Positive IPHA Tests	0	4	1	0	5
IPHA Titers	0	1:256 1:128 1:8	1:256	0	N/A
Percent Positive (Overall)	0	6.06%	2.00%	0%	4.31%
Rodents Surveyed by Species (% Positive)					
CA Deer Mouse (<u>Peromyscus californicus</u>)	0	11(0)	8(0)	0	19(0)
Deer Mouse (<u>Peromyscus maniculatus</u>)	0	34(2.9%)	33(3.03%)	0%	77(2.5%)
Desert Woodrat (<u>Neotoma lepida</u>)	0	1(0)	0	0	1(0)
Dusty-Footed Woodrat (<u>Neotoma fuscipes</u>)	0	8(25%)	8(0)	0	16(12.5%)
Meadow Mouse (<u>Microtus californicus</u>)	0	2(50%)	0	0	2(50%)
CA Ground Squirrel (<u>Spermophilus beecheyi</u>)	0	3(0)	1(0)	0	4(0)
Kangaroo Rat (<u>Dipod. vs heermanni</u>)	0	5(0)	0	0	5(0)
CA Pocket Mouse (<u>Perognathus californicus</u>)	0	2(0)	0	0	2(0)

TABLE 9

Carnivore Serological Surveys for Plague

Vandenberg AFB, California, 1979-82

	1979	1980	1981	1982	Totals
Total Carnivores Surveyed	24	14	8	3	49
Positive IHA Tests	3	1	1	2	7
Positive Coyotes	3	1	1	2	7
IPHA Titers	1:256 1:64 1:128	1:2048	1:256	1:256 1:256	
Percent Positive (Overall)	12.50%	0.89%	12.50%	66.66%	14.29%
Carnivores Surveyed by Species (% Positive)					
Coyote (<u>Canis latrans</u>)	18(16.66)	14(0.89)	7(14.28)	3(66.66)	42(16.66)
Badger (<u>Taxidea taxus</u>)	3(0)	0(0)	1(0)	0(0)	4(0)
Bobcat (<u>Lynx rufus</u>)	3(0)	0(0)	0(0)	0(0)	3(0)

TABLE 10
Ground Squirrel Burrow Surveys for Fleas
Data Collection by Year for 16 Areas (SSSP)*
Vandenberg AFB, CA 1977-1980

Area	Survey Years			
	1977	1978	1979	1980
1	--	--	--	--
2	--	--	--	
3			--	--
4			--	--
5	--	--	--	--
6	--	--	--	
7			--	--
8			--	--
9	--	--	--	--
10	--	--	--	--
11	--	--	--	--
12		--	--	--
13		--	--	--
14		--	--	--
15		--	--	--
16		--	--	--
Total Areas Surveyed	7	12	16	14

"--" Indicates data was collected during period.

*SSSP = Stationary Site Survey Plot

TABLE 11

VAFB Ground Squirrel Burrow Surveys for Fleas
at Sixteen (16) Stationary Sites (SSS)

Number of Each Flea Species Identified by Year, 669 Surveys, CY 1977-1980

Species	Number of Fleas by Year				
	(1) 1977	1978	1979	1980	77-80
<i>Diamanus montanus</i>	900	783	2245	1950	5878
<i>Hoplopsyllus anomalus</i>	68	23	107	65	263
<i>Echidnophaga gallinacea</i>	12 ⁽²⁾	0	0	0	12
<i>Pulex simulans</i>	0	4	3	2	9
<i>Monopsyllus wagneri</i>	0	1	0	1	2
<i>Malaraeus telchinus</i>	0	0	0	2	2
<i>Malaraeus</i> sp.	0	0	2	1	3
<i>Opisodasys (keeni) nesiotus</i>	0	0	1	1	2
<i>Atyphloceras</i> sp.	0	0	0	1	1
Totals	980	811	2358	2023	6172

(1) Data collected for 5 months, May-Sep 77; data collected over 12 months for subsequent years.

(2) 9 of 12 from stationary survey site 1.

TABLE 12

BMDP7D Descriptive Statistics

All Species Fleas Collected, 16 Areas, Partially Adjusted** Data
 VAFB, CA, 1977-1980

Area	Code	Total Fleas Collected	No. Surveys	Mean No. Fleas/ Survey*	%DM	%HA	Study Years
S1	1	630	43	14.651	89.84	4.76	77-80
S2	2	368	29	12.690	97.01	0.27	77-79
S2A	3	456	30	15.200	96.49	3.73	79-80
S2B	4	883	33	26.758	94.68	2.49	79-80
S3	5	392	63	6.222	96.68	3.57	77-80
S4	6	210	29	7.241	91.43	2.85	77-80
S4A	7	317	29	10.931	95.90	2.84	79-80
S4B	8	624	31	20.129	95.51	4.17	79-80
S5	9	392	40	9.550	89.27	8.12	77-80
S6	10	356	37	9.622	96.63	3.09	77-80
S7	11	670	58	11.552	95.23	3.43	77-80
S8	12	369	58	6.362	85.09	6.23	78-80
S9	13	092	19	4.842	59.78	35.86	78-80
S10	14	113	27	4.185	99.00	0.88	78-80
S11	15	289	38	7.605	94.12	4.15	78-80
S12	16	036	06	6.000	97.22	0.00	78-79
OVERALL T-16		6187	570***	10.854	93.68	4.19	77-80

* Survey Flea Index = Ave # Fleas/Survey; Burrow Flea Index can be found by dividing by 15.

**Partially adjusted means surveys with zeros in flea columns were removed from the data set because of: wind conditions during survey, fleas lost in mail, fleas in deteriorated condition precluding identification. No adjustment was made in this dataset for concurrent rodent control measures. In later datasets this adjustment is made.

***Represents 8550 Flagged Burrows.

TABLE 13
VAFB Plague Surveillance Surveys, CY 1977-1980
Numerical and Mean Survey Flea Collections by Species

Species	Year				All Years
	1977	1978	1979	1980	
All Species Fleas Collected	980	811	2358	2023	6172
Diamanus montanus	900	783	2245	1950	5878
Hoplopsyllus anomalus	68	23	107	65	263
Echidnophaga species	12	0	0	0	12
Other Species	0	5	6	8	19
Number Surveys (SSS Type) for Fleas	67	108	160	334	669
Average Number of Fleas Per Survey*					
All Species	14.63	7.51	14.74	6.06	9.23
Diamanus sp.	13.43	7.25	14.03	5.84	8.79
Hoplopsyllus sp.	1.02	0.21	0.67	0.19	0.39
Echidnophaga sp.	0.18	0.00	0.00	0.00	0.02

*Number of Fleas Collected/Number Surveys.

TABLE 14

BMDP7D Descriptive Statistics

All Species Fleas Collected, 16 Areas, 492 Surveys*, VAFB, 1977-1980

Area Code	Sample Size/ No. Surveys	Mean (\bar{X}) No. Fleas Collected/ Survey	STD. DEV.	R.E.S.D. ⁽¹⁾	S.E.M. ⁽²⁾	MAX.	MIN.
1	31	16.194	20.194	20.472	3.627	65	0
2	22	13.091	21.096	18.246	4.498	86	0
3	20	12.000	24.469	16.974	5.471	108	0
4	24	20.250	34.331	30.540	7.008	127	0
5	47	5.383	5.754	5.094	0.839	27	0
6	22	7.773	6.575	6.472	1.402	27	0
7	26	11.154	13.925	13.954	2.731	50	0
8	24	18.458	35.890	28.513	7.326	149	0
9	37	9.189	14.181	12.864	2.331	54	0
10	37	9.622	13.324	12.374	2.190	47	0
11	58	11.552	13.965	12.471	1.834	72	0
12	58	6.362	10.293	7.741	1.352	62	0
13	19	4.842	4.586	4.623	1.052	15	0
14	24	3.125	4.337	3.974	0.885	17	0
15	37	7.622	13.149	10.330	2.162	67	0
16	06	6.000	13.726	12.814	5.604	34	0
OVERALL	492	9.945	17.213	12.934	0.776	149	0

(1) R.E.S.D. = Robust Estimate of the Standard Deviation.

(2) S.E.M. = Standard Error of the Mean.

* Completely adjusted data set including removal of surveys where concurrent rodent control measures were in effect at time of survey.

TABLE 15

VAFB Ground Squirrel Burrow Surveys

Collected Flea Sex Ratio by Species, CY 1977-1980

<u>Category</u>	<u>M:F Sex Ratio</u>
All Species	1:1.088
Diamanus montanus	1:1.099
Hoplopyllus anomalus	1.14:1
Echidnophaga gallinacea	1:1

TABLE 16
Ecology of Fleas from California Ground Squirrel
(Spermophilus beecheyi) Burrows
(Rutledge et al., 1979; Marshall, 1981)

<u>Genus, Species</u>	<u>Common Name</u>	<u>Preferred Temp/R.H.**</u>	<u>Breeding Season</u>
<i>Diamanus montanus</i>	Winter flea or Ground squirrel flea	27°C, 80%	Winter
<i>Hoplopsyllus anomalus</i> *	Summer flea	19°C, 25%	Summer
<i>Echidnophaga gallinacea</i> *	Sticktight flea	Unpublished	Summer
<i>Pulex simulans</i>	None	"	Unpublished
<i>Monopsyllus wagneri</i>	"	"	"
<i>Malaraeus telchinus</i>	"	"	"
<i>Malaraeus</i> sp.	"	"	"
<i>Opisodasys [keeni] nesiotus</i>	"	"	"
<i>Atyphloceras</i> sp.	"	"	Winter

* In coastal regions of California these species are much rarer throughout the year because of the moist cool climate. They require arid climatic conditions to complete the life cycle. On the opposite extreme, D. montanus requires moist conditions to enable breeding and larval survival.

**R.H. = Relative Humidity

TABLE 17

Descriptive Statistics for Populations of Dianthus montanus
and Hoplopyllus anomalus at VAFB, CA.

Mean Number of Fleas Per Survey

	Jul 77	Jan 78	Jul 78	Jan 79	Jul 79	Jan 80	Jul 80
<u>D. montanus</u>	14.46	3.43	5.18	2.25	14.38	2.21	5.17
<u>H. anomalus</u>	0.77	0.57	0.18	0.00	1.15	0.13	0.35

TABLE 18

Plague Serological Tests: A Comparative Analysis (a)

Test	Abbreviation	Year Developed	Serum Component Detected	Rank Order Se*	Plague Screening Rank Order Sp **
Passive and (b) Indirect Passive Hemmagglutination	PHA IHA } IPHA } IDA }	1954 1959	F-1 Ab	2	1
Enzyme-Linked Immunosorbent Assay	ELISA	1979	F-1 Ab both IgG & IgM	1	2
Agar-Gel Precipitin Inhibition	AGPI	1965	Undescribed	4	4
Complement Fixation	CF	1952	F-1 Ab early IgM only	3	3

Rank Order: Best test or of greatest value (1); Least valuable or least accurate (4).

* Se = Sensitivity = Probability that an animal with a positive plague titer will be classified as having one.

** Sp = Specificity = Probability that an animal without a positive plague titer will be classified as not having one.

(a) Rank ordering of the four tests was accomplished by the author based on the below literature and opinions solicited from Drs A.M. Barnes and T.J. Quan, Vector-borne Viral Diseases Division, Plague Branch, NCDC, P.O. Box 2087, Fort Collins, Co 80521 (303) 221-6400/6429 and Dr B.C. Nelson, VCBS, California Dept Health, Berkeley, CA 94704.

(1) World Health Organization. Passive hemagglutination test. Expert Committee on Plague, 4th Report. Technical Bulletin No 447; 1970: p5-25.

(2) Marshall JD et al. Comparison of the reliability and sensitivity of three serological procedures in detecting antibody to Yersinia pestis (Pasteurella pestis). Appl Microbiol 1972; 24:202-204.

(3) Chen TH et al. Studies on the immunization against plague II. The complement-fixation test. J Immunol 1952; Vol 68: p 147-158.

CONTINUATION TABLE 18

(4) Cavanaugh DC et al. Application of the ELISA technique to problems in the serological diagnosis of plague. Bull Pan Am Hlth Organ 1979; 13: 399-402.

(5) Williams JE et al. A comparison of serological tests for detecting antibody to plague. Bull WHO 1976; 54: 3513.

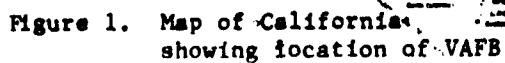
(6) Ray JF, Kadull PJ. Agar-gel precipitin-inhibition technique for plague antibody determinations. Appl Microbiol 1965; 13: 925-930.

(b) The PHA test is used as a high specificity screening test on sera of wild mammals (rodents and carnivores). If the PHA is presumtively positive then a second PHA and an IPHA test is run for confirmation. The IPHA test identifies false positive tests which are potentially possible in animals with antibody titers against Pasteurella sp and/or Y. pseudotuberculosis or Y. enterocolitica.

TABLE 19

Goals for a Comprehensive Plague Epidemiological Surveillance Program

1. Describe the wild mammal and flea populations of the area to include seasonal and geographical patterns [isodemic mapping (IM) and time series analysis (TSA)] and to delineate high risk areas (ANOVA).
 2. Obtain a measure of plague prevalence in wild mammals on the area by temporal and spatial patterns.
 3. Describe the area human population with patterns of activity while living, working or playing there[(IM) and (TSA)].
 4. Describe the area in terms of serving as an ecological niche for sylvatic plague and assess the current and future risk to humans considering expanded operations or changes in range management.
 5. Study effectiveness of and establish priorities for plague preventative control measures (coyote, rodent, flea control; education; surveillance; vaccination) for the area.
 6. Draft an operational contingency plan for control of vectors and protection of the public health during any future sylvatic plague epizootic on the area.
-

[illegible]

VANDENBERG AIR FORCE BASE California

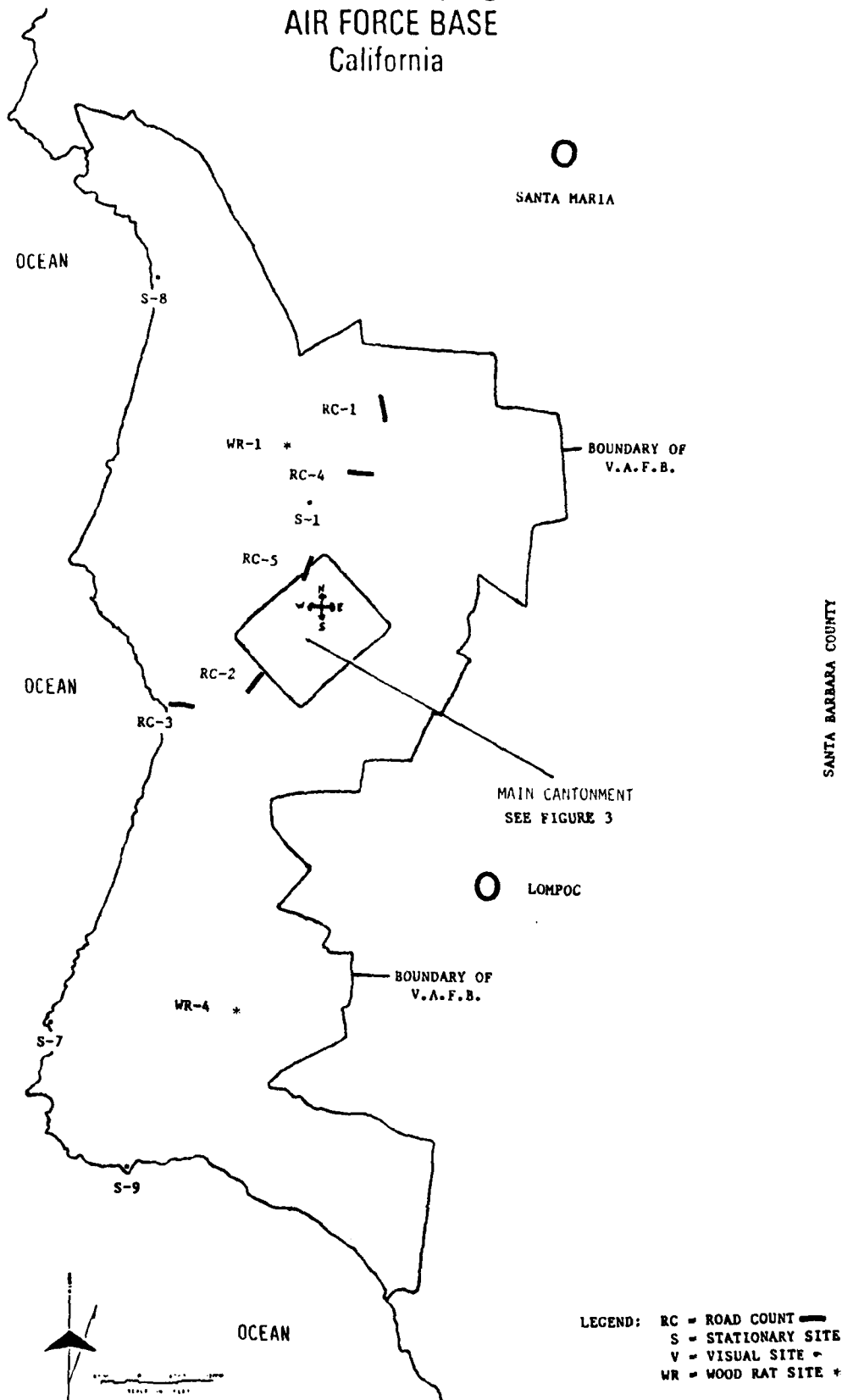


FIGURE 2. Map of Vandenberg AFB Showing Main Cantonment and Survey Locations Outside Cantonment.

VANDENBERG **AIR FORCE BASE** California MAIN CANTONMENT

Legend:

- Stationary Sites
- Visual Sites
- * Wood Rat Sites

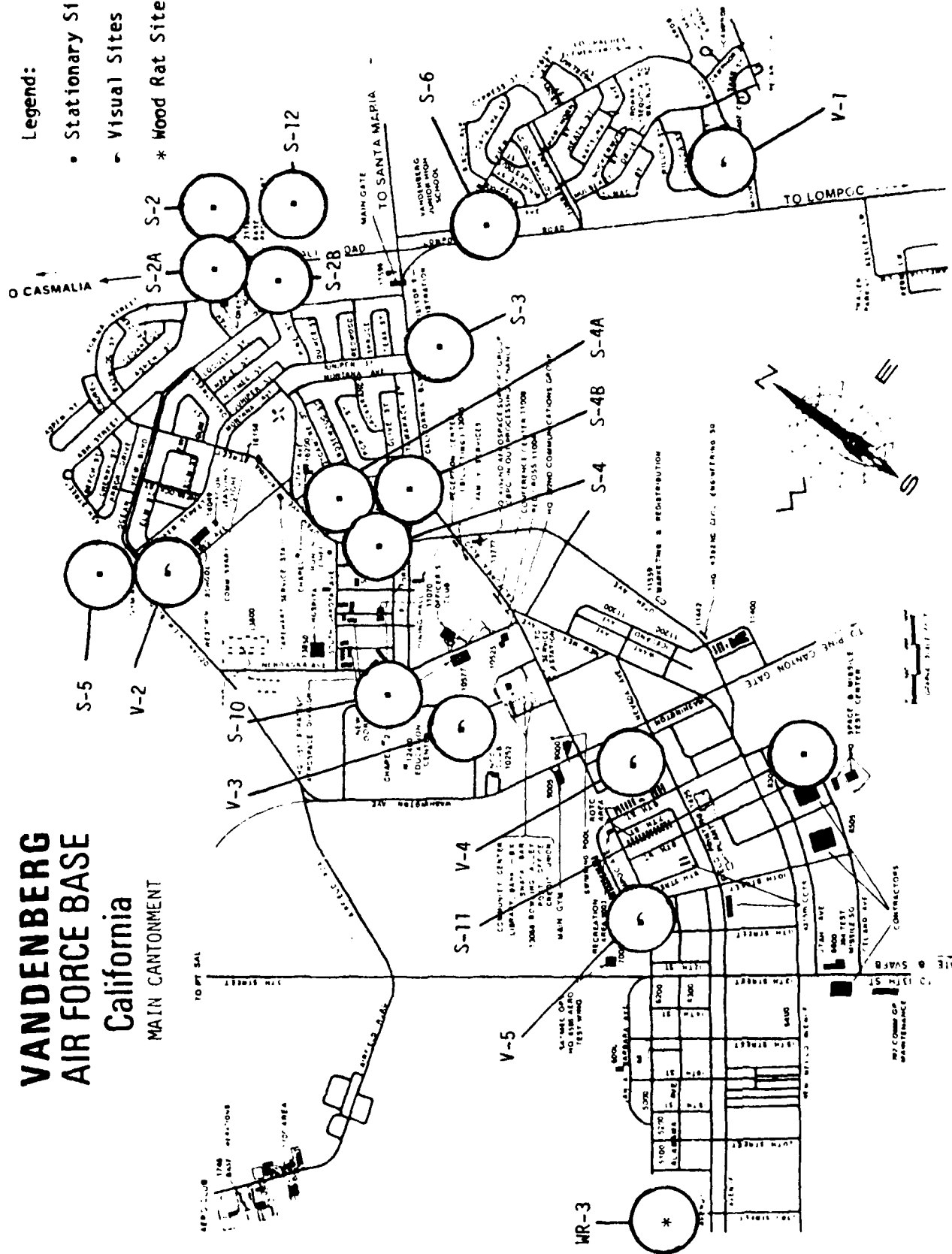


FIGURE 3. VAFB Main Cantonment Showing Location of Survey Sites

SMALL MAMMAL POPULATION SURVEY FOR PLAGUE DETECTION

1. PLACE, CITY, OR TOWN Vandenberg AFB, CA 93437		2. COUNTY Santa Barbara		3. INSPECTOR Brown/Schmaeling		4. DATE 26 Nov 79	
5. DESCRIPTION OF PLACE S-1 Golf Course 16th hole						6. ALTITUDE	
7. SIZE OF AREA 50 X 100 yds		8. TIME WEATHER 0845/drizzling		9. RODENT CONTROL none		10. LAST INSPECTION 22 Oct 79	
11. ANIMALS OBSERVED	NONE	FEW	MED	DENSE	V DEN	DAMAGE	INCREASE DECREASE
CALIF. GROUND SQUIRREL	0						0
GOLDEN MANTLE SQUIRREL							
CHIPMUNKS							
GRAY SQUIRREL							

ROAD ☒ 15 MIN

12. WOODRAT LODGE INSPECTION				13. GROUND SQUIRREL BURROW PREVALENCE			
ABANDONED				ACTIVE		ABANDONED	
ACTIVE	RECENT	OLD	RE-OCCUPIED	TOTAL	ECTOS. POS.	TOTAL	ECTOS. POS.
				16	55	4	
14. ROAD COUNT		PLACE OR NAME		DISTANCE TRAVELED		TIME SPENT	
FROM				END		END A.M. P.M.	
TO				START		START A.M. P.M.	
ROAD USED				TRAVELED		AMT. HRS MIN	

15. REMARKS (SUSPECT BEHAVIOR, DISAPPEARANCE, PREDATORS, ETC.)

Plague found in blood sample from a coyote shot in this area.

Fleas, pool V4-2389: 140/104 *Diomedes montanus*12 *Hesperomys sonoriensis*discarded: 140/154 *Diomedes montanus*

16. DEAD ANIMALS, DISPOSAL

SPECIAL NOTE: (Item 11 "Animals observed") Make subjective estimates of animal abundance on the following basis:

- A. None
- B. Few: Occasional animals seen
- C. Med: Animals scattered or perhaps one small colony
- D. Dense: Individuals frequently seen
- E. Very dense: Large numbers "running all over the place"

FORTRAN Coding Form

0596 611

EXHIBIT 3

Plague Surveillance: Survey Inspectors, 1977-1980

<u>Name</u>	<u>Code</u>
SSgt Tyree	01
A1C Schmaeling	02
A1C Bickford	03
SSgt Sharrock	04
SMSgt Forsythe	05
Sgt Thompson	06
A1C Greer	07
A1C Galen	08
Sgt Brown	09
SRA Mendes	10
SRA Kwasnica	11